

The University of Chicago

A STUDY IN THE LOCALIZATION OF
ORGAN-FORMING AREAS IN THE
CHICK BLASTODERM OF THE
HEAD-PROCESS STAGE

A DISSERTATION SUBMITTED TO THE
FACULTY OF THE DIVISION OF THE
BIOLOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF ZOÖLOGY
1934

By
MARY ELBERT RAWLES

Private Edition, Distributed by
THE UNIVERSITY OF CHICAGO LIBRARIES
CHICAGO, ILLINOIS

Reprinted from
THE JOURNAL OF EXPERIMENTAL ZOÖLOGY
Vol. 72, No. 2, January, 1936

The University of Chicago

A STUDY IN THE LOCALIZATION OF
ORGAN-FORMING AREAS IN THE
CHICK BLASTODERM OF THE
HEAD-PROCESS STAGE

A DISSERTATION SUBMITTED TO THE
FACULTY OF THE DIVISION OF THE
BIOLOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF ZOÖLOGY

1934

By
MARY ELBERT RAWLES

Private Edition, Distributed by
THE UNIVERSITY OF CHICAGO LIBRARIES
CHICAGO, ILLINOIS

Reprinted from
THE JOURNAL OF EXPERIMENTAL ZOÖLOGY
Vol. 72, No. 2, January, 1936



A STUDY IN THE LOCALIZATION OF ORGAN- FORMING AREAS IN THE CHICK BLASTODERM OF THE HEAD-PROCESS STAGE¹

MARY E. RAWLES

Hull Zoölogical Laboratory, The University of Chicago

NINE FIGURES

This investigation was begun with the idea of locating the areas in the chick blastoderm of the head-process stage which possess the capacity to produce heart and liver. The frequent duplication of these organs which was observed in transplants of the entire pellucid area to the chorio-allantois (Willier and Rawles, '31 a) would seem to indicate that the material from which they take their origin is bilaterally placed in the early blastoderm. To acquire some definite knowledge as to the extent of this material in antero-posterior and medio-lateral directions, it was planned to divide the blastoderm into a number of median and lateral pieces, each of which could be transplanted to the highly vascularized chorio-allantoic membrane of older host embryos in order to test their potencies. Obviously, in effecting such an analysis, data relative to the location not only of heart and liver but of many other embryonic organs would be obtained, and it was therefore considered perhaps preferable at this time to construct first of all a general map showing the position, as far as possible, of the various organ-forming areas in the blastoderm as a whole, and then to follow this analysis further by the use of appropriate cuts designed to show the limits of any one of these areas.

¹ The major portion of this work was carried out during an appointment as research assistant to Dr. B. H. Willier, under a grant to The University of Chicago from The Rockefeller Foundation. Certain additions have been made while holding a similar appointment at the University of Rochester.

This paper is concerned with the first of these analyses, namely, a general mapping of the organ-forming areas in the head-process blastoderm. A map constructed in this way represents, of course, the potentialities of a particular part of the blastoderm and not necessarily the normal fate or prospective value. While the map is regarded as being at present in a preliminary stage, nevertheless certain of its features seem striking enough and interesting enough to justify its presentation at this time. A preliminary report of this work was presented before the American Association of Anatomists at the annual meeting in Philadelphia in March, 1934 (Rawles and Willier, '34).

This study was suggested and outlined originally by Prof. B. H. Willier, under whose direction the work has been carried on. It is a pleasure to acknowledge this indebtedness, and to offer him my most appreciative thanks for his help and interest. For the delineation of figures 5 to 9 I am indebted to Mrs. Helen Rearwin Bassett.

EXPERIMENTAL PROCEDURE

The experiments consisted in dividing the head-process blastoderm into eighteen pieces, six median and twelve lateral. Record was kept of the degree of development of the head process and embryos were classified as early, medium, or late in this regard. In the early stage (fig. 1), the head process ranges from invisibility to approximately 0.24 mm. in length, and the area pellucida averages 1.9 mm. in width, 2.8 mm. in length. The stage with a distinct rod-like process 0.24 mm. to 0.6 mm. long is termed medium (fig. 2); at this time the area pellucida averages 2 mm. in width and 3 mm. in length, and the head-fold has not yet appeared although often barely indicated in 0.6 mm. processes. When the late head-process or head-fold stage (fig. 3) is reached, the head-fold, as the name implies, is present, the node has regressed extensively, and the area pellucida while remaining of the same approximate width as earlier has increased to 3.4 mm. length. In this paper,

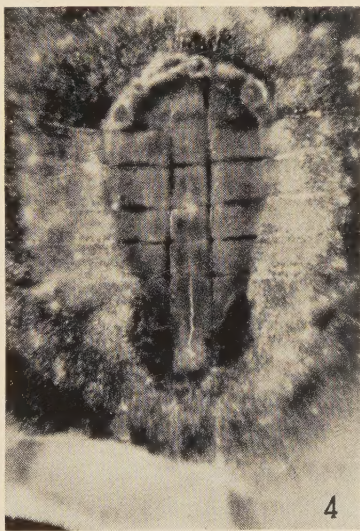


Fig. 1 Photograph of an early head-process blastoderm (fixed in Bouin's fluid, stained with Ehrlich's haematoxylin). $\times 16$.

Fig. 2 Photograph of a blastoderm of the medium head-process stage (fixation and staining same as in fig. 1). $\times 16$.

Fig. 3 Photograph of a living blastoderm of the late head-process or head-fold stage of development. $\times 16$.

Fig. 4 Photograph of a living blastoderm (medium head-process stage) prepared for the isolation of the separate pieces (see p. 275). $\times 16$.

grafts of the medium head-process stage will be discussed primarily and other stages will be referred to when pertinent. Grafts were obtained over operative periods extending from April 22 to June 12, 1931, and from February 9 to June 15, 1933. White Leghorn eggs were used for both hosts and donors. Hosts were incubated at a temperature of 38 to 39°C. for a period ranging from 8½ to 10 days. By this time the chorio-allantoic membrane is exceedingly well vascularized and it is comparatively easy to locate by candling two or three good sites for the transplants without placing them too close together. Donors were incubated for from 18 to 20 hours at the aforementioned temperature. In the majority of instances the head-process stage was attained within this period. The operative method developed for chorio-allantoic grafting by Professor Willier was used with slight modifications, the procedure being as follows: the contents of the donor egg are dropped into a small sterile pan containing warm saline solution (0.9 per cent) in sufficient quantity just to cover the surface of the yolk where the blastoderm is located. With sharp fine scissors the blastoderm is cut from the yolk and transferred in a broad-mouthed pipette to a Petri dish containing Locke's solution (0.9 per cent saline was used in some cases) kept at a temperature of 39°C. by means of an automatic electrically controlled warming stage placed under a binocular dissecting microscope. With its dorsal surface uppermost, the blastoderm is spread out on the bottom of the dish. By pipetting away most of the liquid judiciously, it can be made to lie perfectly flat—an important prerequisite. The blastoderm is now examined carefully with the microscope. If no abnormalities are apparent and if the desired developmental stage has been attained (some phase of head-process formation) then the following measurements are made with an ocular micrometer: length of process, length of area pellucida, and also its width at the node level. The primitive pit is used as a center from which all measurements are made. So far the procedure is more or less preliminary. The chief aim from now on is to get the blastoderm sectioned

into the desired number of pieces preparatory for transplantation. In the earlier experiments the cutting was accomplished by the use of a very fine and delicate instrument—Bowman's iris needle. Later, glass needles made with exceedingly fine points, like those used by Professor Spemann² in his work with amphibian embryos, were tried and found to be admirably adapted for making the isolations on the delicate chick material. With these the cuts can be made not only more quickly and easily but also more accurately. It was soon found decidedly helpful to make preliminary impressions with the needles, outline the cuts as it were, without actually cutting through (fig. 4). This simple precaution facilitates accuracy in making measurements by preventing the troublesome curling of the edges which invariably follows when the blastoderm is cut through.

In the chief series of experiments, the blastoderm was divided by five transverse and two longitudinal cuts into eighteen pieces, two lateral and one median at each of six levels, A to F (fig. 5). One transverse cut passes through the primitive pit, two others are made anterior to this 0.26 to 0.4 mm., and 0.5 to 0.9 mm. respectively. Similarly, posterior to the pit two transverse cuts are made at 0.28 to 0.4 mm. and 0.56 to 0.94 mm. distance respectively. Two longitudinal cuts made 0.1 to 0.18 mm. to the left and to the right of the pit separate the median pieces from the two laterals. In another series which has yielded numbers of grafts furnishing useful data the blastoderm was divided into eleven pieces. Having accomplished the cutting and measuring in accordance with certain aforementioned precautions the chief concern is to get each of the numerous pieces safely implanted upon the host membrane. To prevent any chance of intermingling only one piece is freed at a time. This is rather easily done by simply retracing with the fine needle the impression previously made. Very fine steel beading needles sealed into glass handles were found useful for trimming away the opaque areas at the

² When Professor Spemann visited The University of Chicago in 1931 he very kindly demonstrated the making and use of these needles.

periphery. A tiny bit of the opaque area was always left attached to the lateral and the extreme anterior and posterior pieces. Purposely no definite or regular order of removal was followed so that no one level was always the first or the last to be transplanted. With an especially prepared glass pipette of very fine bore, the isolated piece is picked up from the Petri

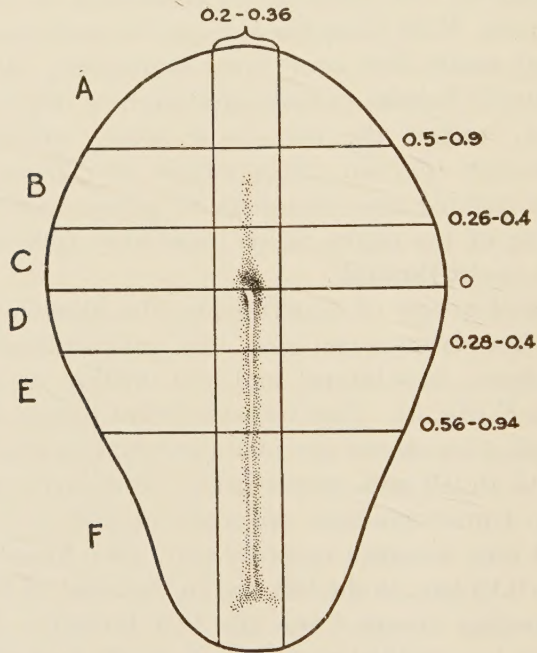


Fig. 5 Diagram illustrating the method of sectioning a blastoderm into eighteen pieces (compare fig. 4). Letters A to F indicate the six transverse strips, each of which is separated into a median and two lateral pieces. Measurements in millimeters. $\times 27$.

dish along with a tiny drop of the solution and transferred to the highly vascularized chorio-allantoic membrane of the prepared host embryo. The transfer is made through a small window sawed in the shell with a small hack-saw blade. Throughout the operative procedure, care was taken to prevent undue contamination. All instruments, containers, towels etc., were sterilized.

After a period of 8 or 9 days' growth on the membrane the grafts were removed from the host, fixed in Allen's B 15, embedded in paraffin, sectioned at 6 or 7 μ , and stained with Heidenhain's iron hematoxylin.

THE DATA

In carrying out this study on the localization of the various organ-forming areas more than 500 grafts have been obtained, of which 388 have been examined histologically. Data from 216 of these, represented in the summary table, form the basis for the construction of the accompanying maps (figs. 7, 8, and 9). The remaining rather large number represent variations in the isolation which exclude them from the type described and figured, although the data have been most valuable in throwing light upon the extent of the various organ-forming areas. Data from the eleven-piece series particularly are used often in the following sections for comparison. As far as possible, grafts from adjoining pieces of a single donor were selected for study since the data supplied by these might be regarded perhaps as most reliable. In no case were grafts from all eighteen pieces recovered from a single donor; the greatest number from any one blastoderm was twelve. Rather frequently, however, six to nine grafts were obtained from one donor. At every level the median pieces yielded grafts more frequently than the corresponding laterals. Levels B, C, and D, gave grafts more frequently than E, which in turn had a higher frequency than F or A. In consequence of this difference in the capacity to produce grafts, many more grafts than needed were obtained from the high frequency levels, during the time that a representative number was being accumulated from those regions with a low or lower frequency. The fact, further, that there is such a great individual variability among grafts makes it necessary to examine quite a number from any one particular location to be able to determine its maximum differentiation with any certainty.

The various structures present in the grafts were identified by their cytological and histological constitution which are not

considered further as they have been described in several papers by Professor Willier and his students.

Level A. The anterior-most region of the area pellucida

The transverse cut which separates level A from the remainder of the pellucid area was made from 0.5 mm. to 0.9 mm. anterior to the primitive pit—the average distance being approximately 0.7 mm. Accordingly, this cut would fall 0.1 to 0.2 mm. anterior to the end of the process and pass through that region of the blastoderm where normally the head-fold forms. The purpose of placing the cut here was to test the potency of this region, quite anterior to the process, which supposedly takes no part in the formation of the embryo proper. Other than the aforementioned transverse cut which separates level A from the next level B, two longitudinal cuts one on each side of the median line 0.1 to 0.18 mm. lateral to the pit separate this region further into a median and two lateral portions. Figure 5 will serve to show how both transverse and longitudinal cuts were made. Of 184 cases where the host remained alive, in thirteen cases only, two median, seven lefts, and four rights, did the membrane at the transplantation site show any perceptible thickening. Thinking that the small size of these pieces as isolated might be the cause of the low frequency exhibited, another series of transplantations was attempted in which the entire level was grafted as one piece instead of being separated into a median and two lateral sections. The result was that nine of the twenty-five living hosts showed a scarcely perceptible thickening of the membrane at the transplantation site. While the difference between the two series may result partly from the fact that the graft site was more carefully scrutinized in the second series, yet probably the size of piece is a factor in persistence and growth of transplants at this level. Another variation in the method of isolation, which has bearing on this question of size of piece, was made in this manner: the entire strip A was divided into two pieces either into halves or into two pieces of unequal size. Five of fourteen such cases

showed some growth, all of which, interestingly enough, came from pieces larger than a third.

The transplants are well vascularized and apparently in good condition but showed no intensive growth and had differentiated but slightly (see table). The only structure recognized as originating from the donor ectoderm is a stratified epithelium, possibly epidermis. It is difficult to distinguish it from the chorionic ectoderm which often becomes quite thickened and stratified, even cornified. Epithelial pearls of chorionic origin are rather common. Since this region of the blastoderm, at the time of isolation, is practically mesoderm free with the exception perhaps of a few wandering mesoderm cells which have come in from the lateral edges, it is not surprising that no typical mesodermal derivatives occur. The grafts all show some slight proliferation of mesenchyme, however, which may be just a reaction of the host mesenchyme to the grafted piece. Occasionally a condensation of the mesenchyme is seen, which becomes quite fibrous in character and is then strikingly like smooth muscle.

The most consistent and characteristic constituent of these grafts is a small epithelial tube or tubule having a low almost cuboidal epithelium with rather dark staining nuclei. More often than not, the cells of these tubules are vacuolated, sometimes extremely so. Hunt ('31) regards such epithelial tubes as endodermal in origin and finds them characteristic of the grafts obtained from the level of the area pellucida anterior to the node in the primitive streak stage. Stein ('33) finds the same structure in her grafts of a similar region both in the head-process and primitive streak stages. Since they occur in grafts from which the endoderm was removed, she suggests an ectodermal origin. The present work can throw no light on the origin of these prevalent epithelial structures; they have been found to occur at any or all levels of the blastoderm, particularly when the transplant shows weak growth. Their unspecific character suggests an arrested differentiation of either or both ectoderm and endoderm. The cytoplasmic vacuolation indicates that degenerative changes

Summary table showing the frequency with which structures differentiated in the grafts

LEVEL OF BLASTODERM	NO. OF GRAFTS STUDIED	NOTOCHORD	CENTRAL NERVOUS TISSUE	FORE BRAIN	MID BRAIN	HIND BRAIN	NEURAL TUBE	GANGLIA	EPIPHYSIS	EYE	EAR	SENSORY EPITHELIUM	ANTERIOR GUT	STOMACH	INTESTINE	EPITHELIAL TUBULES	RESPIRATORY TRACT	THYROID	LIVER	HEART	MESONEPHROS	WOLFFIAN DUCT	SUPRARENAL	PRIMORDIAL GERM CELLS	SPLERN	CARTILAGE	BONE	SMOOTH MUSCLE	SKELETAL MUSCLE	SKIN	FEATHER GERMS	STRAPIPED EPITHELIUM	EPITHELIAL PEARLS		
A Median	2															1		1						2								2	1		
A Left	7															3								4								2	2		
A Right	4															1								1								3	2		
A Whole	9															4		2						4								4	4		
A $\frac{1}{2}$ * and $\frac{1}{2}$ *	5															3								3								2	3		
B Median	18	2	18	17	4					15		5	8	5		6	7	5								14	2	4	1	13			1	2	
B Left	14	13	9	1						4		4	4	3		10	2	4		3	10			4		10	2	5	1	8	5		2	4	
B Right	10	9	8	1						3, 4T		2	5	2		7	2	6		5	7			4		8	1	6		9	2		1	2	
C Median	16	15	16	6	5						2, 1?	8	6	6		12	2	2				4				15	5	6	9	12			4	4	
C Left	11	8	1	1							1	2	2	1		8	2	1, 1?		4	9			4		9	2	3	1	10	4		1	1	
C Right	14	8	3	1							1	4	3			12	3	1, 1?		2	11			3		13	5	6	3	2			2	1	
D Median	16	12	16		4?	11	16					6	5	12		2	2				11	6			2	13	8	10	13	10	1		1	2	
D Left	11		4									3	2	10		2			3	9	3			2		6	3	5	1	6	2		2	2	
D Right	10											1							2	9			1, 1?			5	1	4		4	2		1	2	
E Median	13											9				3					1					8	2	11		7	2		1	1	
E Left	12		1?									6	4							2	1			3		7	1	7	1	9	6		2	2	
E Right	8											2?	3							2	1					3	1	5		4	4		1	1	
F Median	15											5	12			7									1T	11	8						5	8	
F Left	8																																8	4	
F Right	4																																	4	4
F Whole	5											4	2			3										1T	4							3	2
F $\frac{1}{2}$ * and $\frac{1}{2}$ *	5											3				3										2T								1	1

T = Trace.

are occurring. The strong tendency for both of these germ layers to form tubules in chorio-allantoic grafts is very noticeable.

By far the most interesting structures, observed in 37 per cent of these grafts, are the so-called primordial germ cells. They are seen either singly or in clusters among the mesenchyme cells or in spaces, perhaps lymph spaces, so commonly seen in chorio-allantoic grafts. Their large size as compared with the surrounding cells of the mesenchyme makes them stand out conspicuously. According to Swift ('14) the primordial germ cells of the chick arise extra-embryonically from the germ wall endoderm, in a crescentic area located in the extreme anterior region of the area pellucida. Since the transplants described here included either most or a part of this germ crescent the presence of the germ cells is rather to be expected. Also it has been shown previously by Dantschakoff ('32) that these cells, which she calls entodermal wandering cells, will persist and multiply when the germ crescent (Keimsichel) is isolated and transplanted to the chorio-allantois or to the 'Keimscheibe.'

A typical entodermal structure which at the time was rather unexpectedly met with in this level was thyroid. In one case in which the transverse cut was made 0.18 mm. anterior to the end of the process and the level transplanted without further division, a small but rather typical thyroid appears. Two other cases nearly as far anterior also show it, one a transplant of the whole level, the other a left lateral piece, both being cut 0.08 mm. to 0.1 mm. above the end of the process. These three grafts are otherwise quite typical of the level, containing only the structures which have been so far described.

It is interesting for comparison here to mention without giving details, a group of twenty grafts in which the transverse cut was made progressively nearer to the process tip, the two longitudinal cuts remaining unchanged. As the tip of the process is approached and reached a noticeable increase occurs in both frequency and size of graft. Also the median pieces give a higher percentage of grafts and larger ones than

the corresponding laterals. Thyroid, forebrain and eye become the characteristic structures. Thyroid has appeared further anterior than the other two—its anteriormost limit has been already mentioned. In median pieces forebrain has occurred as far anterior to the end of the process as 0.08 and eye as far anterior as 0.04 to 0.06 mm. A further discussion of these structures will be found in the following section where grafts of level B are described.

Before passing to this next level, it may be helpful to summarize briefly the outstanding features of level A. This region shows weak growth and very little capacity for differentiation. Epithelial tubes, the cells of which often appear vacuolated, and epithelial pearls are the most characteristic structures other than the primordial germ cells which persist and undergo multiplication. Rather common is a stratified epithelium, possibly epidermis.

Level B. The forebrain—eye—thyroid region

The portion designated as B is a transverse strip of blastoderm 0.27 mm. to 0.54 mm. in width lying 0.26 mm. to 0.4 mm. anterior to the pit. Two longitudinal cuts made 0.1 to 0.18 mm. to each side of the pit divide this region further into a median and two lateral pieces. As seen from the diagram, this level extends beyond and includes the tip of the process. Sixty-seven grafts were obtained: thirty-three from the middle, twenty-two from the left lateral, and twelve from the right lateral. Compared with the preceding level A we find here a remarkable increase in the capacity for differentiation. Also, a striking difference between median and lateral pieces is noted both in the frequency and size of grafts. Median pieces yielded grafts in 52 per cent of the cases. They showed excellent growth and attained a very large size. The percentage of grafts obtained from the left lateral piece was thirty-two, considerably less than that of the median piece, yet greater than the 19 per cent frequency obtained from the corresponding right lateral. Correlated with this decrease in frequency observed in the lateral pieces is a lower growth rate resulting in their smaller size.

Forty-two grafts were examined histologically, eighteen median, fourteen left, and ten right. As shown in the table, brain and its derivatives, ganglia, the somite derivatives (skin, cartilage, bone, muscle) thyroid, digestive tube, respiratory structures, and various epithelial tubes are common to both lateral and median grafts; chorda and hypophysis (see p. 284) occur only in median grafts; and structures like heart, liver, feather germs, and primordial germ cells appear only in the lateral grafts. In addition to these rather general observations there are certain features which deserve further attention.

Brain is by far the most conspicuously and extensively developed constituent and occurs usually in the form of vesicles. These are irregularly shaped, often branched, and are filled ordinarily with static blood, frequently causing very abnormal distentions of the walls. Although the predominant component of both median and lateral grafts, it is in the median that the greatest proliferation as well as the maximum differentiation is attained. The greater size of the median grafts bears this out. Of the laterals the left grafts show more nervous tissue than the rights and are of larger size. In fact it would not be going too far to say that the size of the graft is a reliable index of the amount of nervous tissue present. In addition to the smaller brain vesicles of the lateral grafts unorganized masses of nervous tissue are seen scattered irregularly throughout the graft, which contain numerous small lumina lined with ependymal cells giving to the whole an exceedingly tubular appearance. This effect is heightened by the existence of numerous epithelial tubes not only in the lateral grafts but in the median as well. Some of these are interpreted as representing a type of sensory epithelium; they seem decidedly more ectodermal than endodermal in character, others have been identified as digestive tract; anterior levels (oral cavity, pharynx, oesophagus) and stomach levels (proventriculus and ventriculus). Many others remain unidentified owing to their variable non-specific epithelia and the fact that neither their connections nor the condition of the surrounding mesenchyme offers any clue relative to their origin.

That the brain at this level is principally forebrain, we judge from the frequent association of the forebrain derivatives, eye and epiphysis, rather than from its specific histology. Mid-brain is less frequently represented. Its specific histology—alternate nuclear and fiber tracts—makes its identification rather certain. Its occurrence also in grafts of level C, leads us to infer that the transverse cut separating levels B and C passes through the prospective mid-brain material.

The appearance of notochord in but two grafts is rather surprising since all of these transplants when isolated contained a part of the head-process or chorda anlage. Its absence from the graft has had no apparent effect on the development of the brain.

The medio-lateral difference in amount and differentiation of brain tissue is found to hold also for eye development. This organ is present in 83 per cent of the median grafts where it attains its maximum differentiation. In three cases the sensory layer of the retina is very well differentiated; in one of these a lens has formed. In the lateral grafts, eye is represented by the pigmented layer. The sensory layer is no doubt present but not sufficiently well differentiated to be recognized as such. Although some pigmented layer was found in seven right grafts, four of these show only the barest trace, confined to a very few cells. In the left grafts the quantity is much greater, although found in only four cases. While the frequency per cent is unusually high on the right side the numbers with which we are dealing are too small to make the difference between the two sides significant statistically. The degree of differentiation is unquestionably superior, however, on the left side.

Epiphysis appears in both lateral and median grafts. It is associated with that region of the forebrain which contains the characteristic choroid plexus. There are no marked medio-lateral differences exhibited in quantity or differentiation, but it does occur more often in the lateral pieces.

Hypophysis has not been found in any of these grafts, but in another experimental series (the eleven-piece series) in which

the cuts were made similarly except that level B was more inclusive antero-posteriorly, it has been identified in two cases in median grafts. Certainly it does not differentiate very commonly from the head-process blastoderm (compare Stein, '33).

Characteristic of this level in company with forebrain and its derivatives is thyroid. Its occurrence laterally as well as medially at this stage has been discussed by Rudnick ('32). The present experiments corroborate hers in this respect, and in addition, due to the further division of the anterior region of the blastoderm by the use of more transverse cuts, give us a more definite idea of its location. Although appearing occasionally in the contiguous levels A and C, growth and differentiation are both noticeably inferior. It is probable that the transverse cuts separating A from B and B from C happened to pass through the extremities of the presumptive area or field. Further experiments are necessary to validate this indication. Certainly, in level B, thyroid reaches its maximum development. As far as differentiation goes, no medio-lateral difference is detected, yet frequency of occurrence is surprisingly high on the right side. This was noticed for eye, and is likewise true for liver. These exceptionally high frequencies exhibited by structures in the right grafts at this level perhaps deserve some comment. At every other level except A, where no medio-lateral differences seem to exist, the tendency is for structures to appear more frequently in the left grafts. This holds also for many other grafts examined which have included either all or part of this same area. In one other series however, the tendency for liver and thyroid to appear more frequently on the right side has been observed. Since the material with which we are dealing is exceedingly variable, and the number of grafts entirely too small to make the differences between the two sides significant, we must refrain from ascribing too much importance to them.

The rather intimate association of liver and heart which was described in earlier experiments (Willier and Rawles, '31 a and b) is again noted here. The occurrence of these two

organs in lateral grafts only, suggests that the material from which they take their origin is bilaterally placed at this stage. Liver has appeared once in a median graft from a donor of an early process stage, in which the median strip was cut rather wider than usual. Also a trace of cardiac muscle has been observed in one median case which would fall in the same category. Such exceptions are of interest in that they indicate that the anlagen of these organs are barely outside of the median strip as cut in these experiments.

Feather germs have not been found in the median grafts although skin figures prominently and in places is exceedingly well differentiated. In the lateral pieces their distribution is scanty, never more than two or three in any one graft. The bone observed at this level is membrane bone; it is not abundant. Skeletal muscle is scarce in the median and left grafts and has not been found in the rights.

Interesting here for comparison is a group of grafts obtained from a series of isolations in which levels A and B were left as one instead of being separated by a transverse cut. Just as in the present series, the region was divided into a median and two lateral pieces. Thirty-five of these grafts have been examined (eleven median, fifteen left, and nine right). In view of the slight capacity for differentiation exhibited by level A, we would not expect its addition to produce any marked effect. According to expectations the structures identified are the same ones listed for level B. With the exception of hypophysis (the two cases already referred to) and some cartilaginous bone, no new structures were observed. One feature worthy of mention is that the lateral grafts showed better growth, due, no doubt, to an increase in size of transplant. The medians again were very large but no larger apparently than those obtained from the B medians alone.

While the experiments with which this paper is primarily concerned were not designed to limit the organ-forming areas, nevertheless certain variations in the cuts which have occurred from time to time do throw light upon this point. For example, a series of isolations already referred to in the

preceding section, in which the transverse cut separating level A from level B fell progressively nearer the tip of the process, i.e., level A was made to include varying amounts of the anterior part of level B, shows very definitely that forebrain and eye are associated with the extreme anterior end of the process. In twenty such grafts examined (ten median, five left, and five right) forebrain occurs in all of the median pieces and practically all of the laterals that are well incorporated. Eight of these median grafts contain eye tissue; also one left and one right graft. As for the other two medians which did not show eye tissue, the boundary was about 0.08 mm. anterior to the process and in both of these cases eye developed from the contiguous B median piece of the same donor. Eye has not been found farther anterior to the process end than 0.04 to 0.06 mm. (two cases). This agrees with the findings of Clarke ('34) who has performed a series of experiments specifically designed to ascertain the antero-posterior and medio-lateral limits of the eye-forming area in both head-process and primitive streak blastoderms. Both forebrain and thyroid are found further anterior than eye. In one graft, median, a forebrain with a beautifully differentiated choroid plexus occurs as far as 0.08 mm. anterior to the apparent end of the process. Such findings lead us to believe that the presumptive forebrain material extends anterior to the process end (compare Stein, '33). There are not sufficient data to decide how far posteriorly in this level forebrain and its derivatives extend; however, ten grafts have been examined in which the anterior limit of level B chanced to be made 0.06 mm. or more posterior to the tip of the process instead of quite anterior to it, as usual. None of these grafts, median or lateral, interestingly enough, showed either eye or epiphysis (there has been only one case in which eye has ever been observed farther posteriorly). Where the contiguous A median graft was recovered from the same donor (one case) it contained both of these structures, well differentiated.

Regardless of variations in the isolation which from time to time have been many, and regardless of the stage, whether early, medium, or late head-process, if a transplant includes the tip of the process the grafts invariably show a high frequency occurrence of forebrain and its derivatives, eye and epiphysis, and thyroid, along with certain other structures more generally distributed.

Thus in summary, level B may be designated as the forebrain-thyroid strip. The other structures, mid-brain, ganglia, notochord, heart, liver, parts of the digestive and respiratory tracts, skin, feather germs, cartilage, bone, muscle and primordial germ cells will be seen again at other levels. The median pieces give the highest percentage of grafts. These are very large, consisting principally of brain vesicles. Left pieces give grafts more frequently than rights, and they are usually larger. In both laterals much unorganized brain tissue appears, in addition to small brain vesicles. Nervous and various epithelial tubes are abundant. Heart, liver, feather germs, and primordial germ cells develop only from lateral pieces.

Level C. The hind-brain—ear region

The transverse strip of blastoderm constituting level C extends from the primitive pit anteriorly for 0.26 to 0.4 mm., the average width being 0.3 mm. From figure 5 it is seen that this portion includes the anterior half of the node plus a good part of the process itself, although the anterior cut is always well behind the tip. The variation in extent anteriorly is due mostly to the varying lengths of the head-process of the different donors. Two longitudinal cuts pass through the blastoderm as usual on either side of the pit from 0.1 to 0.18 mm. separating this level into a median, left, and right portions.

Here as in level B the median piece yields grafts more frequently and larger grafts than either of the laterals. Of the laterals the left gives grafts more often than the right and they have a tendency toward a larger size. The per-

centage of grafts obtained was the following: median 68, left 39, right 32. Compared with the preceding level the per cent has increased for each part of this strip. This increase in yield is due no doubt to the presence of the anterior half of the node.

Forty-one grafts have been examined histologically, sixteen from median pieces, eleven and fourteen from left and right pieces respectively. The data are recorded in the summary table. By far the majority of structures such as brain, ganglia, internal ear, skin, cartilage, bone, muscle, portions of the digestive and respiratory tracts, various epithelial tubes and primordial germs cells are found in both median and lateral pieces. Mesonephros and chorda appear in the median grafts only, while heart, liver, thyroid, and feather germs appear in the lateral pieces.

As in the preceding level brain is the most conspicuous and extensively developed constituent, particularly in the median grafts, where quantitatively and qualitatively it exhibits its maximum differentiation. With the exception of three grafts, it assumes a vesicular form, yet, the vesicles do not reach the enormous proportions seen in the corresponding piece of level B and identified as forebrain. The ventricles are smaller and the walls considerably thicker. Sometimes the whole gives the appearance of a collapsed rather than an expanded vesicle. In six grafts mid-brain is identified. Its appearance is the same as in level B. The remaining vesicles are no doubt hind-brain, although its identification is necessarily based upon resemblance to the form of the normal (thick floor and thin roof) and its association with internal ear, rather than upon a specific histology. In five cases it seems very definitely hind-brain.

Judging from the amount and appearance of brain tissue in the lateral grafts the primordium of this organ is not as extensive laterally at this level as at level B. These laterals show much less nervous tissue per graft, in fact nearly half of the right grafts show none at all. Of the lefts all but three show some. In three rights and one left, small pieces

of mid-brain have been identified. Due to the presence of ear, hind-brain has been identified once in both laterals. Usually, however, the brain is too diffuse and unorganized to make identification certain as to region. The formation of numerous small lumina lined with ependymal cells, in the masses of unorganized brain tissue and giving the tubular aspect so marked in the preceding level, is very much less noticeable.

Ganglionic cells are rather common and in the median grafts the ganglia are often quite large.

With one exception the median grafts contain notochord: a tremendous increase in frequency compared with level B.

This is the only level from which internal ear has differentiated. Found in both median and lateral grafts, it consists of an expanded tube of sensory epithelium surrounded by a cartilaginous capsule. A large ganglion, presumably the acoustic, is quite closely associated. Unless this relationship of capsule and ganglion exists, identification is not certain. In two median grafts and one left lateral, ear has been definitely identified. The left case incidently presents the best differentiation. In one other median and one right graft the epithelium seems identical with that of the cases just mentioned yet the cartilage-ganglion association is lacking.

As in level B, so at this level there exist many tubes of what seems to be a type of sensory epithelium. Undoubtedly some of these represent auditory epithelium of a lower grade of differentiation. In general, epithelial tubes are rather common at this level of the blastoderm. While some have been classed as digestive (stomach and anterior levels) and respiratory, still others remain unclassified due to their non-specific and variable character.

The quantity of skeletal muscle in the median grafts has increased noticeably. It is quite abundant and beautifully differentiated. The associated cartilages are very much larger than those seen in the median grafts of B. Also, cartilage bone which was not observed there is found here in three cases. Feather germs have not been observed although

skin appears in the majority of these grafts. The laterals are not especially different from those of the preceding level as regards the development of somite derivatives. Bone and skeletal muscle have not developed in the right grafts and feather germs are very sparsely distributed.

Cells identical in structure with primordial germ cells have been observed in one median graft. In view of the reputed germ cell origin such an occurrence is very puzzling. Otherwise this particular graft is typical.

In two cases only does the mesonephros of this level show extensive growth—one of these in places is distinctly metanephros-like. In two cases a small clump of mesonephros-like tubules is the only indication of kidney. We shall see a marked increase in the development of this organ in the next level posteriorly.

The thyroid observed in the lateral grafts is far from typical, exhibiting very scanty growth. In regard to the left graft containing the one case which seems more typical, the protocol shows that the piece when isolated was cut rather wider than usual for the length of head-process exhibited, consequently the presence of thyroid in it can be explained by its encroachment upon level B, the specific thyroid level.

The structure occurring most consistently in the lateral grafts, even more frequently than nervous tissue, is heart. Its frequency has increased on the left side from 71 per cent at level B to 81 per cent at C, and on the right side from 70 per cent at B to 78 per cent at C. On the whole the masses of cardiac muscle at level C are somewhat larger. Liver closely associated with cardiac muscle occurs much more frequently on the left (36 per cent) than on the right (14 per cent), just the reverse of the condition observed at level B, and an excellent illustration of the variability of the material under graft conditions.

To summarize, many structures like mid-brain, ganglia, notochord, digestive, respiratory and various epithelial tubes, heart, liver, thyroid perhaps, primordial germ cells, and the generalized structures, bone, cartilage, skin, muscle, feather

germs recur at this level. Others as mesonephros, ear, hind-brain make their first appearance. Ear and hind-brain are the characteristic structures. Graft frequency reaches its peak at this level in the order of median, left and right. Compared with level B the median grafts have decreased somewhat in size following the decrease in size of brain vesicles. Cartilage and skeletal muscle have increased considerably. Also notochord shows a tremendous increase in frequency. Brain is less extensive laterally. Heart is the most prominent lateral structure.

Level D. The neural tube—mesonephros region

The portion of the blastoderm designated as D is bounded anteriorly by a transverse cut passing through the primitive pit and posteriorly by a similar cut varying from 0.28 to 0.4 mm., the average width being about 0.3 mm. The longitudinal cuts passing 0.1 to 0.18 mm. to either side of the pit divide the area into a median and two laterals as in the levels already described. A total of fifty-one grafts was recovered, twenty-six median (59 per cent), fifteen left (27 per cent), and ten right (20 per cent). As in the preceding levels the capacity to produce a graft is strikingly greater in the median than in the lateral transplants. Of the latter the left is superior in this respect. Thirty-seven of these grafts have been examined histologically, sixteen median, eleven left, ten right, and the contained structures listed in the summary table. Briefly, these data show that notochord, suprarenal and spleen develop only from median pieces. Central nervous tissue, mesonephros, bone, cartilage, muscle, various epithelial tubes, skin and feather germs occur both medially and laterally, although nervous tissue, mesonephros, respiratory epithelium and skeletal muscle have not been identified in the right laterals. Heart, liver and primordial germ cells occur laterally only.

The median grafts resemble those of levels B and C in that every one contains central nervous tissue, but differ from them in respect to the quantity present. Here nervous tissue no longer forms the bulk of the graft. Usually it takes the

form of rather solid masses which may or may not exhibit lumina and which resemble neural tube much more closely than brain. In eleven cases the whole topography is decidedly like neural tube. In four cases a small brain-like ventricle is completely filled with a very dense blood clot. These possibly represent hind-brain. One such configuration occurs in a graft with a very normal appearing neural cord and is continuous with it. In two grafts the nervous tissue is too diffuse and unorganized to suggest any particular region. While most of the nervous tissue is more cord-like than brain-like both no doubt exist at this level. Certainly there is no reason for thinking that a cut chosen arbitrarily to pass through the pit should separate the two primordia absolutely. Notochord is well differentiated in twelve grafts. Ganglia are quite common.

The decrease in nervous tissue noted in the lateral grafts of level C is even more marked here. Not one of the rights contains even a trace; ganglia being the sole representatives of the central nervous system. Four of the eleven lefts however contain a small quantity but not sufficiently well organized to classify as to region.

Heart and liver are distinctly lateral. Liver appears rather infrequently and never in large quantities although typical histologically. Heart is the one organ which seems as well if not better differentiated on the right side than on the left. Its frequency of occurrence reaches a peak at this level increasing to 90 per cent on both sides. In view of the relatively small numbers considered, too much stress should not be put upon this percentage—nevertheless heart is unquestionably the predominant constituent of the lateral grafts of this level. In two grafts (rights) it is the only structure present, with the exception of a few vacuolated tubules.

Epithelial tubes are relatively less abundant than at either of the preceding levels. The marked decrease in those which are presumably sensory is perhaps correlated with the decrease of nervous tissue. No respiratory tubes were identified on the right side. Some of the digestive tubes in the median and left grafts are intestine and represent levels posterior to

the stomach region, while others are identified as belonging to the stomach level. The anterior gut levels—oral cavity, pharynx, oesophagus—found rather abundant in level B, and to a certain extent in level C, are no longer evident. There yet remain some epithelial tubes in nearly every graft that are too unspecific to allow identification.

Skeletal muscle has not occurred in the right grafts and once only in the lefts. It is abundant in all but three of the median grafts where also the cartilages are large and common. Skin is less conspicuous in the median than in the laterals. Feather germs are found in grafts from both laterals: less in number from the right pieces. In one median graft several have developed within a skin vesicle. This one case is regarded, however, as too exceptional to record on the map.

The majority of the median grafts contain mesonephros. At this level it reaches its maximum development. In six cases wolffian duct is associated. In three cases portions are distinctly metanephros-like. The mesonephros of the three left grafts is very small yet all of the components are represented. No trace of this organ was seen in the ten right grafts studied yet evidence obtained from a slightly different series of isolations including much of the same area shows unquestionably that this region does have the capacity to produce mesonephros. In the series referred to, a strip of blastoderm was cut approximately the same width as level D and divided into the same three parts, the only difference being that the cut instead of passing through the pit, passed just below it. It eleven right grafts studied from such an isolation, one contains a small mesonephros; two others have clusters of mesonephric tubules but no glomeruli. On the basis of this finding mesonephros will be included in the map of the right section of level D.

Suprarenal is typically differentiated in one-half of the median pieces, but it has not been observed laterally.

A variation in cuts which is of interest here includes an earlier series of isolations in which the node level was not bisected, i.e., no transverse cut passed through the pit, but the

cuts were made 0.1 to 0.2 mm. above and below the pit and the strip divided as usual into median and laterals. Such an isolation is very nearly equivalent to the combined levels C and D, perhaps not quite as extensive antero-posteriorly. Forty-one grafts have been examined, fifteen median, fourteen left, and twelve right. As expected, the structures developed in this series are identical with those found in the corresponding regions of C and D except for two additional organs, gonad and pancreas. Each has occurred in two of the fifteen median grafts, and in addition pancreas has developed in one of fourteen left grafts and in a graft from the left half of a strip cut about 0.1 mm. posterior to the pit. It is strange that neither has shown up in the other series. Gonad would be expected in grafts of the posterior half of the node (D medians) on the basis of the frequent occurrence of mesonephros and suprarenal in that area and has so been recorded on the map.

In summary, the median pieces of level D yield grafts more frequently than either of the laterals and they invariably show better growth. Of the laterals the left gives grafts more often and these contain more structures. Nervous tissue at this level is neural tube principally and possibly some hind-brain; it is chiefly median. Heart is the most conspicuous lateral structure, present in all but two grafts. Mesonephros and suprarenal are exceedingly well differentiated in the median grafts. Structures here which have not occurred before are neural cord, suprarenal and spleen. The others, notochord, ganglia, heart, liver, various epithelial tubes, skin, cartilage, bone, muscle, feather germs, mesonephros, and primordial germ cells have been observed in either one or both of the preceding levels.

Level E. The region just posterior to the node

This strip of blastoderm contiguous with level D lies quite posterior to the node region. Its anterior boundary varies from 0.28 mm. to 0.4 mm. below the primitive pit. Posteriorly the boundary varies from 0.56 mm. to 0.94 mm. from the pit. The average width is about 0.4 mm. As usual, longi-

tudinal cuts ranging from 0.1 mm. to 0.18 mm. on either side of the pit divide the strip into a median and two lateral pieces. Of 166 transplants (fifty median, sixty left, fifty-six right) where the host remained alive, thirty-five grafts were recovered, thirteen median or 26 per cent, fourteen lefts or 23 per cent, and eight rights or 14 per cent. Compared with the preceding levels B, C, and D, a drop is noticed in the percentage of grafts obtained both laterally and medially. While the decrease in grafts from lateral pieces is small, that from the median pieces is remarkably great. Thus, the conspicuous difference noted previously between the capacity of the median and lateral pieces to yield grafts is not as evident here. Although the highest yield still comes from the median piece, the difference between it and that exhibited by the left pieces is too small to be significant. As at other levels, here also the yield from the right transplants is the lowest and the grafts exhibit poor growth. Correlated with the decrease in grafts obtained from the median pieces is their small size. Heretofore the size differences between the median and laterals have been quite conspicuous. At this level with one exception which happened to come from a left transplant, the grafts have all been small. Certainly no marked differences were detected macroscopically between the median and left grafts, although the rights were consistently less well grown.

Thirty-three grafts have been studied histologically. The data are found in the summary table. Here as in preceding levels certain medio-lateral differences exist which deserve comment.

None of the grafts, median or lateral, show any tissue that is definitely brain or neural tube. One median graft has a trace of what seems to be fiber tracts and ganglionic cells possibly representing central nervous tissue. It is very atypical. Other than this questionable case, which incidentally comes from an embryo just on the border-line between the early and medium head-process stages, ganglia are the sole representatives of the central nervous system. Though not abundant they are large and well differentiated. The gangli-

onic cells resemble those of the sympathetic rather than the spinal ganglia and are more often than not associated with intestine. Skin is common in both median and lateral grafts yet it is far more abundant in the latter, where it is extremely well differentiated. Usually it takes the form of ingrowing vesicles and tubes rather than being confined to the graft surface. One feather germ occurs in each of two median grafts, but it is in the lateral grafts especially that these structures show their maximum differentiation. At no other level are they so numerous or so well developed. Instead of growing on the outside of the graft they develop more often within skin vesicles. Their differentiation is accelerated and exceeds by far that seen in the normal 9- or 10-day feather germ. The cartilages at this level are smaller than those seen at levels C and D with the exception of one left graft, already mentioned, which attained a very large size (5×4.8 mm.) and in which is found quite an over proliferation of this tissue. In this same graft cartilage bone is rather extensively developed; the only time it has been observed at this level. Membrane bone is found in one median and one right graft. No skeletal muscle has been identified with certainty, however, in at least one case there are strands of muscle which undoubtedly represent it in spite of the fact that no cross striations could be detected.

The digestive tube of this level is intestine and beautifully typical with both muscular coats. In the median grafts it is the most conspicuous structure attaining its best growth and differentiation. Very often the intestinal segments are surrounded by lymph cavities resembling a coelom, and it is with such an arrangement that the differentiation and contour are most normal—a fact noticeably true for practically all graft structures. The almost complete absence of unspecific epithelial tubes so common elsewhere is indeed a striking feature of the grafts of this level. Of the lateral grafts, intestine is more typically differentiated on the left side and the segments are longer. On the whole in the lateral grafts skin and feather germs are slightly more prominent than digestive tube.

Primordial germ cells have been observed from grafts of the left pieces only (three cases).

Heart is represented in two left and two right grafts by tiny masses of typical cardiac muscle. Its feeble development here is in striking contrast to the excellent growth exhibited in the three preceding levels. In both cases the right and left hearts came from the same donor blastoderm in which the transverse cut separating levels D and E was made 0.3 mm. posterior to the pit. While these experiments do not limit the heart area posteriorly, the very fact that it never occurs when the cut is made further behind the pit than 0.3 mm. indicates that we are dealing with the posterior-most portion of the heart-forming area or field. The unusually small size of the only four masses of cardiac muscle which ever developed confirms this interpretation.

The decrease in frequency of occurrence of mesonephros at this level is almost as notable as that of heart. Although it occurs medially and laterally, the median grafts here by no means show the extensive development of this organ seen in the corresponding grafts of the preceding level. The best development of mesonephros happened to come from a left graft whose anterior boundary was 0.28 mm. posterior to the pit. On the right side and in the middle rather meager developments have appeared when the cut was made 0.4 mm. from the pit.

The one case of suprarenal, from a median piece, comes from an isolation where the anterior limit of E was 0.3 mm. behind the pit. It is typically differentiated.

Certain grafts, obtained from making variations in the cuts, are important in giving us some hint as to how far posteriorly within level E certain organ primordia such as heart and mesonephros, for example, extend. As already pointed out, heart has appeared only when the transverse cut was at least 0.3 mm. behind the pit while small developments of mesonephros have been found when the cut was 0.1 mm. further posteriorly. Such cases suggest that these two embryonic areas do not extend far into E. Still others throw more light on this point. One set of grafts constituting a variation in isolation comes from transplanting in its entirety a strip of

blastoderm 0.35 mm. wide cut approximately 0.4 to 0.6 mm. posterior to the pit. It was thought that the developmental capacity might be more completely realized in such undivided pieces. In six grafts examined, neither heart nor mesonephros appeared, although other structures as intestine, skin, feather germs, cartilage, were abundantly differentiated. The number of grafts is small yet it indicates, it seems, that heart as well as mesonephros is more apt to occur if a region slightly nearer to the pit is included. This point is nicely illustrated in a large series of isolations in which the cuts were made in such a way as to include the posterior half of level D and the anterior half of level E. As usual the strip was divided into a median and two laterals. Of thirty-two grafts studied (twelve median, nine left, eleven right) mesonephros and cardiac muscle appear considerably more often than from level E as described. About half of the median grafts and one-third and one-fourth of the left and right grafts respectively, show mesonephros, while half of both laterals show small masses of cardiac muscle. In addition to these organs nervous tissue which was not found in grafts of level E appears in very small quantities in half of the median and a third of the left grafts. Since a study of the grafts of levels D and E has shown us that D possesses a developmental capacity superior to that exhibited by E, we would expect the partial inclusion of D to bring about just such changes which have been mentioned.

In summary, level E has yielded grafts less frequently than either B, C, or D. The median pieces, especially, show a decided drop in graft frequency; also nervous tissue is no longer present. Structures occurring medially and laterally are mesonephros, ganglia, skin, cartilage bone, smooth muscle and digestive tube (intestine). Those occurring laterally only are heart, feather germs (see p. 297) and primordial germ cells (left). Suprarenal is the one structure developing only in median pieces. Intestine, skin, and feather germs are excellently differentiated and are characteristic structures of this level.

Level F. The posterior portion of the streak

Level F includes the hindermost portion of the area pellucida and extends from the posterior limit of level E, i.e., from 0.56 to 0.94 mm. posterior to the pit, to the opaque area. In some cases the transverse cut separating E and F was made as far as 1.0 mm. or more posterior to the pit. As usual, the area was divided into a median and two lateral parts, see diagram (fig. 1). F includes more of the actual area of the blastoderm than any other level. Its length is about 1.1 mm., which takes in approximately the posterior half of the streak. Of 151 transplants where the host remained alive, in twenty-seven cases (twelve median, eight left and four right) some reaction was visible at the transplantation site. The majority of these reactions, which take the form usually of rather large vesicles filled with a lymph-like fluid, can scarcely be called grafts as the amount of tissue differentiated is so very minute.

In about one-half of the medians and one-fourth of the lefts, however, careful scrutiny revealed a tiny pinkish speck on the walls of the vesicles. In the remaining cases, marked questionable at the time of removal from the membrane, tissue growth could be detected only after a microscopic examination of the sections. The growth exhibited by this region of the blastoderm is strikingly feeble.

The best differentiation here as in the preceding levels (A excepted) comes from median pieces. Of fifteen grafts obtained and examined histologically ten show epithelial tubes that unquestionably represent posterior levels of the digestive tube with well-developed muscular layers. In six of these the segments although short are structurally quite like normal intestine. In the remaining four the muscular layers are not so completely formed, sometimes being absent altogether from one side. Especially is this the case when the segment develops near the periphery of the graft. Non-specific epithelial tubes perhaps representing an arrested or incomplete development of the gut epithelium occur rather frequently. Vacuolated tubules like those described for level A are abundant. In eight cases small growths of skin have been observed; in five

others there is a stratified epithelium which resembles epidermis yet the underlying mesenchyme remains too undifferentiated to permit its being classified as skin. In one case only has cartilage appeared and this is merely a trace. Other than epithelial pearls occurring in eight grafts the structures just enumerated represent the maximum differentiation obtained from this level.

The lateral grafts are decidedly inferior to the median as regards capacity for both growth and differentiation. Eight left grafts representing a frequency of 16 per cent and four right grafts representing a frequency of 8.6 per cent, were examined. In only two of these left grafts could any tissue be seen macroscopically. The remaining left and all of the right grafts were extremely insignificant looking. Small epithelial tubes, entirely too atypical to classify as representing any particular region of the digestive tract, occurred in three left and one right grafts. This represents the best differentiation attained by these pieces. Occasionally a few smooth muscle fibers were noted. The most common structure is the already mentioned small epithelial tube with vacuolated cells. This type of tubule, it will be recalled, was the most characteristic component of the grafts of level A where likewise growth and differentiation were very feeble. Skin has not been identified in the lateral grafts although a stratified epithelium is found which may be epidermis. It is not clear whether it comes from the ectoderm of the grafted piece or is a development of the chorionic ectoderm. It is found usually on the outer surface of the membrane rather than growing into the mesenchyme as skin often does. As in the median pieces, epithelial pearls are common.

To determine whether or not size of piece was somewhat responsible at this level for the relatively feeble development exhibited, several variations were made in the type of isolation. The area was transplanted as a whole, was divided into halves, each of which was transplanted separately, and finally the cut separating median and lateral regions was omitted first on one side and then on the other so that two-thirds of the

area could be transplanted as one piece. The results from an histological study of ten grafts recovered from such varied isolations are summarized in the table. Excepting the more frequent appearance of very small pieces of cartilage (three cases) the larger transplants apparently give no better results as regards either growth or differentiation, than the median pieces alone.

The four grafts in which cartilage has appeared came from transplants in which the transverse cut was made from 0.66 mm. to 0.74 mm. posterior to the pit. It has not been observed in some twenty odd cases where the anterior boundary of F was further behind the pit than this. If, on the other hand, we bring the limiting cut further forward so that the posterior part of level E is included as was done in another series of isolations, we get at once a considerable increase in the occurrence of cartilage. Likewise, bone, skin, and feather germs, structures rather prominently differentiated in level E, show up repeatedly.

Briefly, the outstanding features of level F are: the region as a whole shows weak growth, the lateral pieces in particular. By far the best differentiation comes from the median transplants which contain approximately the posterior half of the primitive streak. The structures observed in the median pieces are: intestine, unidentified epithelial tubes and tubules many having vacuolated cells, smooth muscle, skin, a trace of cartilage, and epithelial pearls. In the lateral transplants the best differentiation is represented by a few epithelial tubes, many tubules with vacuolated cells, a few smooth muscle fibers, epidermis, and epithelial pearls.

GENERAL DISCUSSION

The foregoing tests of the potencies of small pieces of the blastoderm, through transplantation to the chorio-allantoic membrane, show clearly that each of these isolated pieces possesses a specific developmental capacity which manifests itself in the frequency with which such pieces grow as grafts, the amounts and kinds of tissues and organs which develop in

them, and the degree of differentiation which such tissues and organs attain. These differences are not haphazard, but are related to the axiation of the embryo for they grade in both an antero-posterior and a medio-lateral direction.

The capacity of transplanted pieces to survive and grow under graft conditions and the amount of tissue produced, exhibit conspicuous differences along the antero-posterior axis. The anterior and posterior ends of the area pellucida

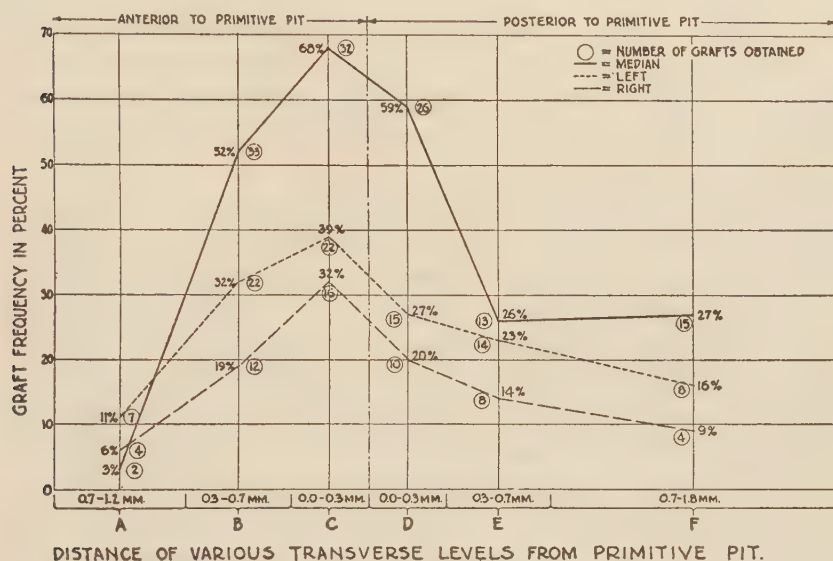


Fig. 6 Graph illustrating the differences in graft frequency obtained from the various isolated pieces of the blastoderm.

yield grafts much less frequently than do those portions including either the node or the head-process and they are invariably smaller. These differences in graft frequency with respect to the antero-posterior axis are graphed in figure 6 in which distance from the primitive pit in millimeters is represented on the abscissa, graft frequency in percentage on the ordinate, for all three transverse pieces of the six levels. The three curves are of the same general form and show clearly that graft frequency rises to a peak at level C, the level

including the anterior half of the node, and declines from this region to posterior levels. The observed curves in ability of the pieces of different levels to grow as grafts appear to express differences in cell activity at different levels. Hyman's observation ('27) on the disintegration of young chick blastoderms in toxic solutions indicates that the node and head-process are physiologically the most active regions at the head-process stage. On this basis it is understandable why these regions grow most readily when isolated as grafts, and also produce the largest amount of tissue. The sizes of the grafts obtained have not been graphed but would yield approximately the same curves as the graft frequencies, with the peak at levels B and C and a decline in both directions from these levels.

The gradation in the quantity of the various tissues and organs produced by grafts with respect to antero-posterior level is also of the same general character. The principal organs appear in the largest quantity in grafts from levels B, C, and D, and decline in amount in both directions (more rapidly anteriorly) from this center, which includes the whole of Hensen's node and the head-process. The most conspicuous example of such quantitative relation to level is furnished by the nervous system, which in grafts of level B proliferates enormously into extensive brain vesicles composing the bulk of the large grafts obtained from this area. From this level posteriorly to the node level the quantity of nervous tissue gradually diminishes, being distinctly less in the posterior than in the anterior half of the node, and behind the node falling off sharply. Thus already 0.1 to 0.2 mm. posterior to the pit, only about half the grafts show traces of nervous tissue, and nervous tissue is never formed in levels 0.3 to 0.4 mm. behind the pit or more posterior. Similar relations are exhibited by cardiac muscle and mesonephros. The former attains its greatest development in level D, posterior half of the node, where very large masses of heart muscle are observed in 90 per cent of the cases and from there declines in quantity rather gradually anteriorly and sharply posteriorly.

Mesonephros similarly is evident in largest quantity in level D and decreases in amount in both directions from this level, showing very scanty development 0.3 to 0.4 mm. behind the pit.

The digestive tube and such general tissues as cartilage, bone, muscle, skin, and feather germs display much less quantitative relation to the antero-posterior axis. They occur in most of the pieces but nevertheless do exhibit some axial relationship. Thus cartilage is absent from level A, is present in a small amount in level B, shows its best development in C and D, declines in amount again in E, and is very rarely seen in grafts from level F. Feather germs and skin seem to occur more often and in larger quantities from the level just back of the node, while feather germs are completely lacking from levels A and F.

The primordial germ cells differ from all other structures of the embryo in that they reach their highest frequency in level A, where comparatively large numbers occur in 37 per cent of the grafts. Although they were present in but few grafts posterior to this region, nevertheless they seem to grade posteriorly to a level 0.7 mm. behind the pit on the left side and 0.3 mm. on the right side. These findings indicate that the horns of the germ-cell crescent of Swift should be extended considerably further posteriorly than he figured them.

Even more noticeable than the antero-posterior gradation is the medio-lateral gradation in developmental potencies of the grafted pieces. At every level except A, the median pieces show the greatest power to produce grafts; this capacity is much reduced in the left pieces and still more so in the right pieces. These differences in graft frequency in the medio-lateral direction are nicely represented in the curves plotted (fig. 6). The frequencies are obviously and consistently higher in the median grafts, distinctly lower in the left grafts, and lowest in the right grafts. These frequency differences between median and lateral pieces and right and left pieces have been checked by statistical methods and found to be significant. The power of pieces to survive and grow as grafts must then already differ with respect to the symmetry of the embryo at the time when they are isolated.

Differences in the size of grafts obtained from median and lateral pieces closely parallel the frequency differences. As a rule the largest grafts come from the median transplants, the smallest from the right lateral pieces.

A medio-lateral relation appears also with regard to the amount of recognizable differentiated tissue in the grafts. The median grafts contain the greatest amount of differentiated structures and this fact indeed is responsible for their large size; while the small right grafts contain the least amount. For example, median grafts at the head-process level are characterized by large conspicuous forebrain vesicles while the lateral grafts, particularly those from the right side, produce reduced and often comparatively minute forebrain vesicles. Further back at level D, the median grafts contain considerable nervous tissue while the left laterals display only traces, the right laterals no nervous tissue at all. Median grafts of the node level, further, contain large quantities of cartilage with which is associated an abundance of beautifully differentiated skeletal muscle, while in the left lateral grafts cartilage and muscle are much smaller in amount and are still more decreased in the right lateral grafts, where skeletal muscle has not been identified with certainty.

Certain structures, notably central nervous system and eye, show a distinct medio-lateral gradation with regard to degree of differentiation attained as well as size and frequency. Eye develops from median pieces with a high frequency, 83 per cent, and the degree of differentiation attained by all its components corresponds with that of the normal. In the lateral pieces, on the contrary, not only is the frequency of eye occurrence much lower, but the differentiation is distinctly inferior to that of the control and often the pigmented layer of the retina is the only recognizable constituent. On the right side the amount of pigmented layer is often reduced to a few cells. Similar conditions obtain with regard to central nervous system. This appears in 100 per cent of median grafts, 70 per cent of left grafts, and 50 per cent of right grafts from levels B-D. Its histological differentiation is strikingly more

normal in median than in lateral grafts, in which it frequently occurs as a formless mass, especially in right grafts.

The most striking result of the present study is the demonstration that particular structures develop only from rather definite regions of the blastoderm, in short, that the organ-forming areas are localized to a remarkable extent. So characteristic are some organs of particular levels that we can speak of an eye-forming level, a thyroid-forming level, an ear-forming level, a mesonephros-forming level, etc. Such localization is well illustrated by the brain and main sense organs. Forebrain develops from level B and from no other region. Eye and epiphysis are also confined to the same level. Mid-brain develops from B and also from the contiguous strip C but from no other levels. Level C produces hind-brain and ear while the following level D gives neural cord. Regions with their anterior margin 0.1 to 0.2 mm. behind the pit develop small masses of nervous tissue too unorganized for identification, while the posterior levels of the blastoderm fail to produce any nervous tissue. Thyroid formation is practically limited to level B. Some other organs are less well localized than those just mentioned but nevertheless show well defined limitation to certain transverse strips of the blastoderm. The mesonephros is confined to a cross strip which includes the whole of D and adjacent portions of C and E. Liver formation can be obtained throughout B, C, and D, and heart formation occurs over a wide region including the zones B-E.

In addition to this relation to antero-posterior cross strips, many organs develop in obvious relation to medio-lateral organization. Some organs form only from median pieces, others only from lateral pieces, and some appear in both lateral and median pieces. Chorda, hypophysis, suprarenal, gonad, and spleen occur only in grafts of median pieces. The only organs whose formation is limited to lateral areas are the heart and liver, although the data of Rudnick ('32) indicate that at an earlier stage (primitive streak) these organs may develop from median pieces. Feather germs also dis-

play a bilateral localization for they have been rarely obtained in median grafts. Skin, although present in the great majority of median grafts, appears to develop better and in greater abundance from lateral grafts. A number of structures occur in all three pieces of a transverse strip. Some of these as thyroid and epiphysis are not correlated apparently with medio-lateral organization but the number obtained is not sufficient to permit any very definite conclusion. Most of the other organs which develop in all three sections of a transverse level clearly occur in far higher percentages in the median pieces and grade off laterally; in this class fall the parts of the brain, the eye, the ear, and the mesonephros. Thus central nervous system occurs in 100 per cent of median grafts, 70 per cent of left grafts, and 50 per cent of right ones. Mesonephros (levels D-E) is produced by median pieces with a frequency of 41 per cent, by left and right pieces with frequencies of 17 and 8 per cent, respectively.

The superior developmental capacity of the left side of the blastoderm as compared with the right has been strongly evidenced throughout these experiments. This is, no doubt, an early manifestation of a similar difference observed in growth and differentiation throughout the normal development of the chick embryo.

When the results are surveyed as a whole it is seen that the levels which produce the greatest quantity and the greatest variety of differentiated structures are the levels B, C, and D which include the head-process and the node. These three levels are capable of producing all of the organs of the embryo. The remaining transverse levels A, E, and F, furnish no structures which cannot be produced by this area alone. According to the work of Hunt ('31) a strip of blastoderm including just the node is totipotent at the primitive streak stage. In the present work with head-process stages it would appear that the totipotent region has widened antero-posteriorly and this may be assigned to the formation of the head-process by the backward growth of the node. This result is in good agreement with the findings of Wetzel ('29) who conceives the

blastoderm in primitive streak stages as made up of sectors converging at the node, which sectors later in the head-process stage straighten out to transverse strips by backward elonga-

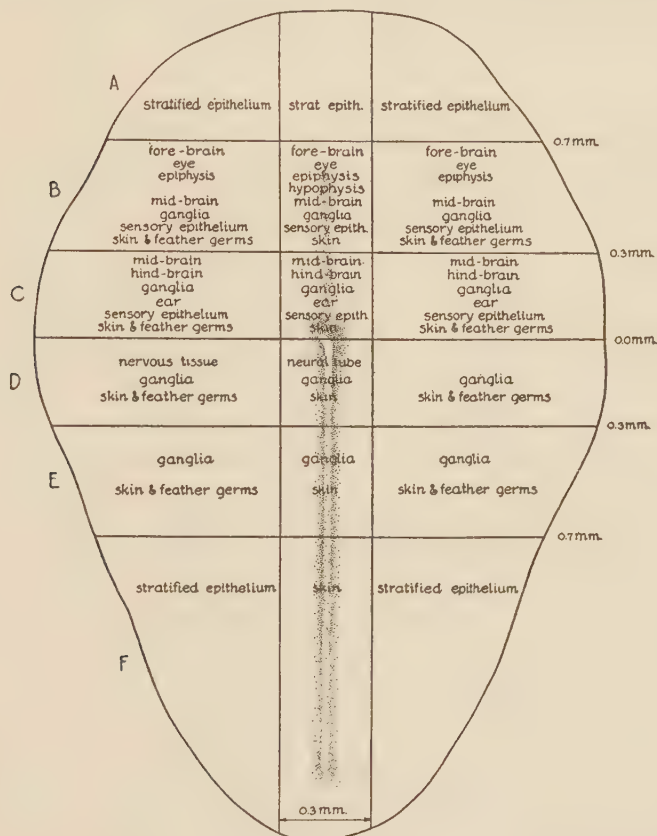


Fig. 7 Map showing the localization of developmental potencies of ectodermal structures in grafted pieces of the head-process blastoderm. The distances from the primitive pit indicated in millimeters are average measurements. $\times 37$. (Prepared by Willier and Rawles.)

tion of the median region. Wetzel's conclusion that the posterior half of the streak plays no role in the formation of the embryo is also supported by the present experiments.

The accompanying maps (figs. 7, 8, and 9) represent an attempt to picture the location of the various organ-forming

potencies in the head-process blastoderm. A different map has been made for each of the germ layers to avoid complication, but it must be clearly understood that the germ layers were not transplanted separately. Each grafted piece con-

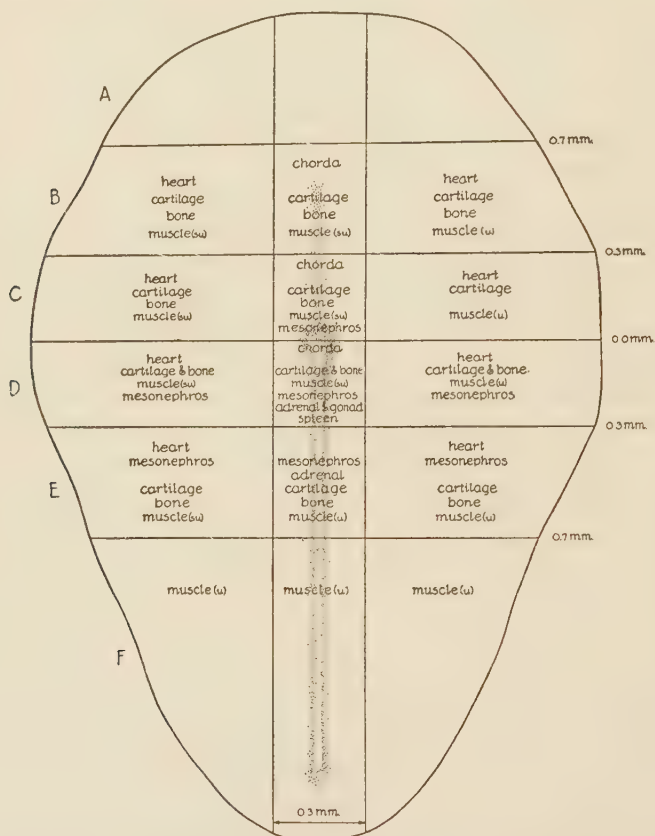


Fig. 8 Map showing the localization of developmental potencies of mesodermal structures in grafted pieces of the head-process blastoderm. S = striated muscle, U = non-striated muscle. $\times 37$. (Prepared by Willier and Rawles.)

tained all three layers, in their normal relationship. It must also be borne in mind that these maps can represent only the prospective potencies of a given area, not its prospective value. Each organ-forming area indicated on the maps presumably represents a field which is capable of producing the

organ in question but only a small portion of which actually forms the organ during normal development (Willier and Rawles, '35).

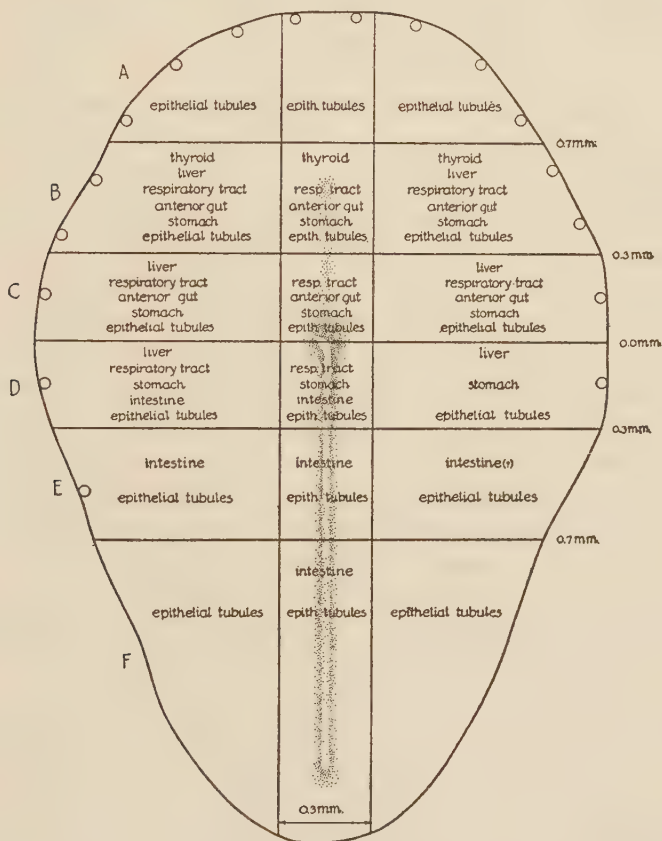


Fig. 9 Map showing the localization of developmental potencies of endodermal structures in grafted pieces of the head-process blastoderm. Circles at margin indicate pieces from which 'primordial germ cells' were obtained. $\times 37$. (Prepared by Willier and Rawles.)

These maps may be compared with other attempts to localize formative areas on the chick embryo. As long ago as 1874 Wilhelm His, reasoning from the mode of formation of the chick embryo, pointed out that the flat undifferentiated blastoderm might be considered as an aggregate of organ-forming

areas, the potentialities of which would be realized through the process of development. His's diagram shows dorsal organs medially located and ventral organs such as thyroid, lung and liver, laterally located. While a certain striking similarity exists between the theoretical concepts of His and the present experimental analysis with regard to some points, His's scheme is based on too simple a ground to justify detailed comparison. More pertinent to the present discussion is the work of Peebles ('04), Kopsch ('27 and '33), and Wetzel ('29). Peebles and Kopsch concluded on the basis of injury experiments that the anlage of the entire head is situated well anterior to Hensen's node at the primitive streak stage, therefore anterior to the anterior end of the head-process at the head-process stage, and apparently included in level A of the present experiments. However, this level entirely fails to yield any head organs when grafted. Even with the addition of level B this region does not represent the entire head but furnishes structures only back to the mid-brain level. It is readily seen that the results here presented do not conform to these schemes of normal development marked out by injury methods; it must be recalled, however, that the data are not comparable because of the differences in experimental stage used and the fact that the present experiments test the prospective potency of the areas in question rather than their prospective significance. These data on the other hand show a more general conformity with Wetzel's ('29) conclusions based on vital staining. The process and node regions, levels B, C, and D, which in the grafts showed an extensive and abundant development of central nervous system correspond in a general way with Wetzel's localization of this system on his map (abb. 120, S. 311). Further his location of the somite region coincides rather well with the region found to yield an unusual abundance of somite derivatives—cartilage, bone, muscle, etc.—namely, the node region, particularly its posterior half. Again, Wetzel found that the posterior portion (approximately the rear half) of the streak forms no axial mesoderm and in the present experiments grafts of this region

(level F) produced practically no mesodermal derivatives. This agreement in results obtained by two very different experimental methods encourages us to believe that some progress has been made in the analysis of the localization of organ-forming areas of the chick.

SUMMARY

1. The chick blastoderm of the head-process stage was divided into as many as eighteen pieces, six median and twelve lateral, and the potencies of each small piece tested by transplantation to the chorio-allantoic membrane; 388 of these grafts have been examined histologically.

2. Each transplant was found to possess a specific developmental capacity which manifests itself in a) the frequency with which it grows as a graft, b) the amounts and kinds of tissues developed, and c) the degree of differentiation attained.

3. Differences in developmental capacity are related to the axiation of the embryo, for they grade in both antero-posterior and medio-lateral directions.

4. It is demonstrated that particular structures develop only from rather definite regions or levels: e.g., the forebrain-eye-thyroid level; the neural tube-mesonephros level, etc.

5. The anteriormost portion of the blastoderm and the portion including approximately the posterior half of the streak reveal slight developmental capacity.

6. Cross strips of the blastoderm which include the head-process and node are capable of producing all the organs of the embryo.

7. At the various levels along the antero-posterior axis many structures develop in obvious relation to the medio-lateral organization. Thus there are organs developing in a) lateral areas only (heart, liver), b) median areas only (chorda, suprarenal, spleen), c) median and lateral areas, but occurring in higher percentages in the median (brain parts, eye, ear, mesonephros).

8. These observations lead to a two-fold interpretation of organ-forming areas in the head-process blastoderm: a) that they tend, some markedly, some less rigidly, to be restricted to a given level or region of the pellucid area; b) that within each area developmental potencies diminish peripherally from a center.

9. The superior developmental capacity of the left side of the blastoderm as compared with the right has been demonstrated.

10. Maps are presented which show the localization of the various organ-forming potencies in the head-process blastoderm.

LITERATURE CITED

- CLARKE, L. F. 1934 Regional differences in eye-forming capacities of early chick blastoderms as studied in chorio-allantoic grafts. *Anat. Rec.*, (Supplement), vol. 58, p. 54.
- DANTSCHAKOFF, WERA 1932 Keimzelle und Gonade IIB. Ganzheit des Gewebekomplexes als Faktor in der Entwicklung der Gonade. *Zeit. f. Zellforsch. u. Mikr. Anat.*, Bd. 15, S. 581-644.
- HIS, WILHELM 1874 Unsere Koerperform und das physiologische Problem ihrer Entstehung. F.C.W. Vogel, Leipzig.
- HUNT, T. E. 1931 An experimental study of the independent differentiation of the isolated Hensen's node and its relation to the formation of axial and non-axial parts in the chick embryo. *J. Exp. Zoöl.*, vol. 59, pp. 395-427.
- HYMAN, L. H. 1927 The metabolic gradients of vertebrate embryos. III. The chick. *Biol. Bull.*, vol. 52, pp. 1-39.
- KOPSCH, F. 1927 Primitivstreifen und organbildende Keimbezirke beim Hünchen untersucht mittels elektrolytischer Marken am vital gefärbten Keim. *Zeit. f. Mikr.-Anat. Forsch.*, Bd. 8, S. 512-560.
- 1934 Die Lage des Materials für Kopf, Primitivstreifen und Gefäßshof in der Keimscheibe des unbebrüteten Hühnereis und seine Entwicklung während der ersten beiden Tage der Bebrütung. *Zeit. f. Mikr.-Anat. Forsch.*, Bd. 35, S. 254-330.
- PEEBLES, FLORENCE 1904 The location of the chick embryo upon the blastoderm. *J. Exp. Zoöl.*, vol. 1, pp. 369-383.
- RAWLES, MARY E., AND B. H. WILLIER 1934 A study in the localization of organ-forming areas in the chick blastoderm of the head-process stage. *Anat. Rec.*, (Supplement), vol. 58, p. 34.
- RUDNICK, DOROTHEA 1932 Thyroid-forming potencies of the early chick blastoderm. *J. Exp. Zoöl.*, vol. 62, pp. 287-313.
- STEIN, KATHRYN F. 1933 The location and differentiation of the presumptive ectoderm of the forebrain and hypophysis as shown by chorio-allantoic grafts. *Physiol. Zoöl.*, vol. 6, pp. 205-235.

- SWIFT, C. H. 1914 Origin and early history of the primordial germ cells in the chick. *Am. J. Anat.*, vol. 15, pp. 483-516.
- WETZEL, R. 1929 Untersuchungen am Hühnerkeim. Die Entwicklung des Keims während der ersten beiden Bruttage. *Arch. Ent'mech.*, Bd. 119, S. 188-321.
- WILLIER, B. H., AND MARY E. RAWLES 1931 a The relation of Hensen's node to the differentiating capacity of whole chick blastoderms as studied in chorio-allantoic grafts. *J. Exp. Zool.*, vol. 59, pp. 429-465.
- 1931 b Developmental relations of the heart and liver in chorio-allantoic grafts of whole chick blastoderms. *Anat. Rec.*, vol. 48, pp. 277-301.
- 1935 Organ-forming areas in the early chick blastoderm. *Proc. Soc. Exp. Biol. and Med.*, vol. 32, pp. 1293-1296.

